The potential of marrow stromal cells in stem cell therapy

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REVIEW

Most tissues tend to contain specialised cells that originate from a small subset of highly undifferentiated, self-renewing elements called 'stem cells' that have the potential to persist throughout adulthood.[1,2] Thus, during normal development these stem cells generate intermediate progenitor cells which are capable of proliferating and differentiating into multiple yet distinct cell lineages. Stem cells derived from immature embryos are capable of differentiating into all somatic cell types[3,4] and those derived from adult tissues have been thought to produce only the cell lineages characteristic of the tissue of isolation.[5]

The bone marrow is the primary site where self-renewal and differentiation of haematopoietic stem cells occurs.[6,7] One subgroup of bone marrow stem cells, bone marrow stromal cells (MSCs), have been shown to possess greater multilinear potential than previously thought. MSCs have been shown to differentiate into osteogenic (bone), chondrogenic (cartilage) and adipogenic (fat) lineages *in vitro*.[8–14] However, several recent studies[12,15–17] have observed that MSCs under strict experimental conditions can differentiate into various cell lineages, including muscle, glia and hepatocytes (Fig. 1).

There appear to be several advantages in using MSCs as opposed to haematopoietic stem cells (HSCs) when considering either cell or gene therapy. First, it has been shown that MSCs are relatively easy to isolate and culture[6,18,19] compared with HSCs which have been observed to be quite difficult to expand in culture.[20–22] Second, only small volumes of MSC are extracted at harvesting,[10,17,23] while larger volumes of marrow are needed in order to obtain adequate numbers of HSCs.[11]

Besides the bone marrow, stem cells can be successfully extracted from both animal and human fetal tissues.[2,4,24–27] Due to the ethical and moral dilemmas this poses, advancement of this avenue into cell therapy could be limited. This, in addition to the fact that MSCs are not restricted to producing specific cell types, namely those from the tissue in which they reside,[28] emphasises the enormous potential of these cells in both cell and gene therapy. This review focuses on the potential of bone-marrow-derived MSCs in such settings, with particular attention to their application in the eye.

Bone marrow stromal cells (MSCs)

Most if not all initial experiments with HSCs incorporated the techniques of either Friedenstein *et al*.[29] or Dexter *et al*.[6] Both methods result in a heterogeneous population of cells that include fibroblasts, fat cells, endothelial-like cells[30–34] and smooth-muscle-like cells.[35–37] These adherent cultures of stromal cells have the advantage of remaining undifferentiated and viable for extended periods for time.[6,17] Despite this, the heterogeneous nature of the culture limits the application of this model due to the difficulty of interpreting the results.

Due to these limitations, a number of subsequent studies incorporated magnetic separation techniques to yield 'purer' colonies of several stromal cell lineages.[38,39] Other studies have utilised additional separation techniques including flow cytometry,[40] relative uptake of acetylated low-density lipoprotein[41] and by the isolation of cellular aggregates.[42]

A number of experiments have focused on the expression of immunological markers that define certain stromal cells in order to isolate a more homogeneous cell population. Work conducted by Perkins and co-workers[43,44] involved the isolation and purification (>95%) of MSCs using the MECA-10 antibody as a reagent for magnetic cell sorting. Similarly, other markers that have been utilised to specifically identify MSCs have included STRO-1, H513E.3, 6.19 and KM16, which identifies fibroblasts, adipocytes, erythroid and endothelial cells.[18,45–49] Other studies have focused on sorting MSCs via the isolation of specific protein-producing cells[50] or via the production of cytokines.[51] All these methods have the additional advantage over conventional MSC cultures that the subcloning of isolated cells typically results in the production of a homozygous cell population.

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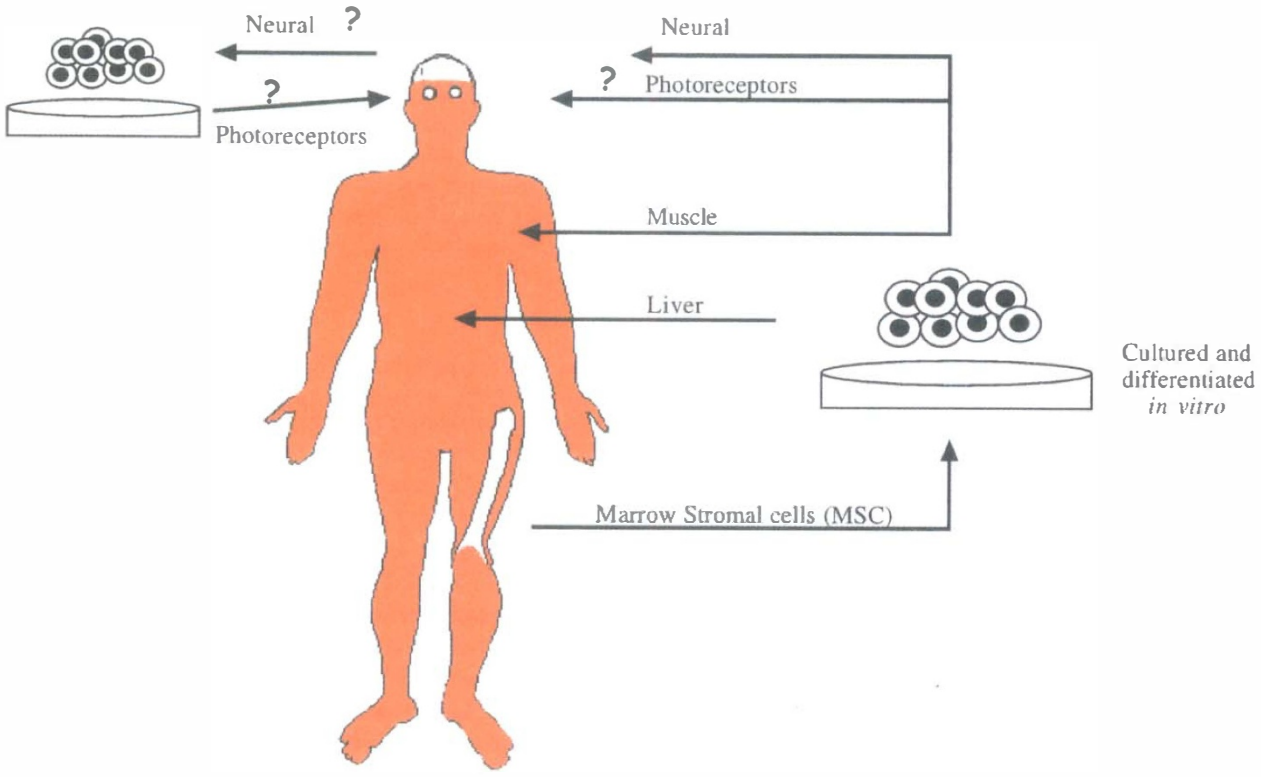

Fig. 1. *Schematic diagram indicating the potential of MSCs in the production of non-haematopoietic tissues. Arrows highlighted with a question mark indicate potential applications only.*

Multilinear potential of MSCs

A number of investigations have found that MSCs are capable of fully differentiating into numerous haematopoietic cell lineages. Initially, MSCs were thought to differentiate into a number of cell types including osteoblasts, chondroblasts and adipocytes, all of which are located in tissues immediately surrounding the bone marrow, but only recently has their differentiation into these specific cell types been elucidated *in vitro*.

Osteoblasts are responsible for synthesising new bone matrix[9,52] and their differentiation *in vivo* is thought to consist of a proliferation phase, a matrix maturation phase and a mineralisation phase.[53] *In vitro* studies using rat and human MSCs have found that a number of agents including dexamethasone,[18,54–58] L-ascorbic acid[59] and β-glycerolphosphate[60] are all essential in inducing MSCs to express an osteoblastic phenotype. Other studies have focused on the culture of cartilage cells from MSCs due to their potential treatment of conditions including degenerative arthritis. Initial studies induced chondroblast formation by culturing MSCs in micromass pellets in the presence of dexamethasone and transforming growth factor-β(3).[61,62] Resulting cultures were shown to develop a multilayered rich morphology whose cells expressed markers typical of articular cartilage.[14,61,62] Diduch and co-workers[63] have also reported that a gellification agent, alginate, seems to induce chondrocyte formation *in vitro*, and when used as a carrier agent, aids in the repair of full-thickness osetochondral defects *in vivo*.

Recent advances in MSC experimentation have enabled the culture of adipocytic cells *in vitro*. Work conducted by Pittenger and co-workers[14] has shown that tripotent progenitor cells can be induced to differentiate into adipocytic cells by treatment with 1-methyl-3-isobutylxanthine, dexamethasone, insulin and indomethacin. Induction was visualised by the presence of lipid-rich vacuoles within cells. However, work conducted by Muraglia *et al.*[64] has found that MSCs lose their adipocytic differentiation with increasing cell doubling.

Potential of bone marrow stromal cells for non-haematopoietic tissues

Neural cell regeneration

Considerable interest in the isolation and expansion of neural progenitor cells exists due to their potential application in the treatment of central nervous system disorders. The majority of these studies have focused on the isolation of stem cells obtained from embryonic and adult animal brains.[2,25,65–68] Results from these studies have shown that isolated stem cells could easily be differentiated into neurones and glia with the removal of mitogens and the addition of serum. Recent *in vivo* investigations have progressed further by successfully transplanting the differentiated stem cells into lesioned brains.[2,27,69,70] Tumour development was absent in transplanted animals and immunohistochemical staining showed that transplanted differentiated stem cells had integrated extensively into the host tissue.

Interestingly, a number of studies using neural stem cells have shown that stem cells in general are not restricted to producing the specific cell types found in their tissue of origin. Suhonen and co-workers[71] observed the differentiation of adult hippocampus-derived stem cells into olfactory neurones, and more recently Bjornson and colleagues[28] showed that genetically labelled embryonic stem cells transplanted into hosts were progenitors to a number of blood cell types including myeloid and lymphoid cells.

There have been only a few studies investigating the multipotential of MSCs for non-haematopoietic tissues. Kopen and co-workers[4] were the first to investigate the theory that MSCs could adopt neural cell fates when exposed to the brain microenvironment. In this study bis-benzimide labelled cells were injected into the lateral ventricle of the brain of cryoanaesthetised 3-day-old mice. Twelve days after the transplant the mice were killed and their brains examined by immunohistochemical staining. Results showed that at 12 days post-transplant, MSCs had migrated throughout the forebrain and cerebellum. No evidence of tumour development was observed. It was noticed that MSCs had migrated and integrated into neurone-rich regions including the Islands of Calleja, the olfactory bulb and the internal granular layer of the cerebellum. There was also evidence to suggest that some MSCs had differentiated into astrocytes and neurones.[4] Additionally, two more recent studies conducted by Sanchez-Ramos and colleagues[17] and Woodbury *et al.*[23] investigated the multilinear potential of MSCs. In both studies human and mouse MSCs were successfully induced to differentiate into neurones under strict experimental conditions.

Bone marrow and liver regeneration

Oval cells are considered to be precursor cells that have the capacity to proliferate and differentiate into hepatocytes or bile duct cells when such cells are prevented from proliferating, usually in response to liver damage.[72] Hepatic oval cells express particular haematopoietic surface markers, including CD34, Thy-1, and c-kit mRNAs,[16,73,74] and previous work by Petersen and colleagues[16] has shown that oval cells and other liver cells, such as hepatocytes, can originate from a cell population in or associated with the bone marrow.

Bone marrow and brain development

The brain is composed of two general cell types: neurones and glial cells. Glial cells play an important physiological role in that they assist in neuronal function and repair neuronal damage due to injury or disease. Glial cells are divided into two subgroups: (1) macroglia, derived from neuroectoderm,[75] and (2) microglia, whose origin remains unclear. A number of studies have shown microglia to originate from neuroepithelial cells[76,77] whereas others argue that they originate from haematopoietic stem cells.[78,79] Thus Eglitis and Mezey[80] conducted a study to investigate whether glia in disease- and injury-free adult brains originated solely from cells present in the brain from the fetal stages of development, or whether there was a migration of cells into the brain from outside the central nervous system (CNS). *In situ* hybridisation techniques were used to detect genetically tagged bone marrow cells in the brains of recipient mice. Results showed that marrow-derived microglia cells were detected in recipient brains 3 days after transplantation, and continued to be incorporated into the brain even up to 70 days after transplantation.[80] Immunohistochemical analysis also showed that the marrow-derived cells were widely distributed throughout the brain, and were detected in the cortex, hippocampus, thalamus, brain stem and cerebellum.[80]

Bone marrow and muscle regeneration

Postnatal repair of muscle fibres is mediated by satellite cells which are located between the sarcolemma and the basal lamina of the muscle fibre, but whose functional capacity is limited by a slow replication rate[81] and a decreasing capacity for self-renewal with age.[82] However, after injury, the numbers of satellite cells observed are much smaller than the number of committed myogenic precursors that populate the muscle fibre.[83] Initial studies by Wakitani *et al.*[84] found that MSCs were in fact capable of differentiating into contractile myotubes under strict experimental conditions *in vitro*. Subsequent transplantation studies conducted by Ferrari and colleagues[85] showed that MSCs were indeed capable of migrating into degenerated sites and fully differentiating into muscle fibres that would then participate in the regeneration process.

Breast tissue regeneration

Immunohistochemical staining methods have identified subpopulations of cells in the mammary gland including terminal end buds (TEB), lateral buds (LB) and alveolar buds that are composed of a heterogeneous collection of cells including stem cells.[86–88] Explant studies have shown that when defined segments of the mammary gland have been excised and transplanted, full regeneration of the mammary gland has resulted.[89] Most of the current applications of stem cell technology in this field are directly involved as part of the chemotherapeutic regimen[90–92] which is now being trialled clinically in breast cancer patients.[93–98] No studies at present have targeted stem cells, including MSCs, for their potential role in the regeneration of lost or disfigured breast tissue. Partial mastectomies that usually result in breast tissue disfigurement could benefit particularly from MSC therapy, and this warrants further investigation.

Keratinocyte tissue regeneration

As in other tissues, stem cells found in the skin (termed holoclones) are the progenitor cells that give rise to fully differentiated cells in response to injury or stimulation,[99] and are thought to account for 1–10% of the epithelial basal cell population.[100,101] Subpopulations of the skin, like the dendritic epidermal cells, have been found to express specific surface markers such as Th-1 which also are expressed by haematopoietic cells.[102,103] Recently, Young and colleagues[104] were able to differentiate MSCs into pure cultures of dendritic colony forming units *in vitro*. Since no other myeloid cell types were identified in derived dendritic colonies it was concluded these cells contribute to the epidermis and afferent lymph where dendritic cells are the principal myeloid cell type.[104] Due to the complex nature of the skin and surrounding structures (e.g. hair follicles) very few studies have investigated the potential application of stem cells in this field.[104,105] Further investigation is required to fully explore their potential role in wound healing, alopecia and skin disorders such as vitiligo.

Retinal regeneration

Retinal failure can be caused by a combination of factors such as exposure to intense light, aging, or genetic factors. Degeneration is often characterised by the progressive death of one or other subset of cells of the retina, such as photoreceptor cells. It has been demonstrated that retinal failure involves programmed cell death, i.e. apoptosis. In spite of the loss of one cell type, function may still exist in the remaining retina and the axons connecting the retina to the brain. Hence photoreceptor or retinal pigment epithelium (RPE) cell replacement may aid in the restoration of some degree of vision.

Therapeutic strategies

Retinal transplantation

Despite the complexities associated with photoreceptor transplantations, several advantages of this therapeutic regimen make it a viable option. First, the retina does not appear to undergo glial scar formation when damaged, which may be due to the fact that retinal cells are capable of regrowing severed axons within the eye or due to their close proximity to their postsynaptic targets.[106,107] Second, the photoreceptor layer of the retina is non-vascularised, which limits most other neural tissues when considered for transplantation.[108] Third, the lack of vascularisation reduces the risk of tissue rejection. This in addition to the fact that very little MHC class I and II expression is observed on photoreceptors, limits their vulnerability to transplant rejection.[109]

Initial studies in animals involved the transplantation of full-thickness retinas which, although remaining ordered and viable, showed a limited ability to integrate with the host retina that was age-related.[110–113] Subsequent studies incorporated the use of retinal cell suspensions which reduced the complexities of the surgical procedure and trauma to the recipient.[114,115] Results from these and other studies showed that the cell suspensions usually formed differentiated rosette structures rather than well-organised layers.[114–117]

One particular study, conducted by Silverman and Hughes,[108] successfully transplanted sheets of photoreceptors from retina that had been gelatin-embedded and vibratome-sectioned. Results showed that transplanted sheets of photoreceptors remained viable for at least 6 weeks, and that the cells were capable of producing visual pigment and thus transduce light.[108] Additional studies since then confirmed this method of photoreceptor sheet transplantation.[118,119] Other studies in turn have focused on the transplantation of fetal cells into hosts. Ghosh and co-workers[120] investigated the long-term effects of full-thickness embryonic retinal transplants in the rabbit. Embryonic neuroretina was harvested from 19-day-old fetal rabbits and transplanted into hosts which were then monitored for a period of 10 months. Results showed that full-thickness embryonic retinal transplants were able to survive without immunosuppression for at least 10 months when positioned with correct polarity.[120] Similar results were also obtained by Sharma and colleagues[121] and Aramant *et al.*,[122] who successfully co-transplanted intact sheets of fetal retina with RPE *in vivo*.

Recently, Woch and colleagues[123] transplanted intact sheets of rat fetal retina with RPE into recipient animals suffering from photoreceptor degeneration. In particular, retinas with attached RPE from 14-day-old rat fetuses were excised, embedded in alginate and then transplanted into the subretinal space of recipient animals, which were then monitored over a period of 10 months post-transplant. Results obtained showed that this transplantation technique restored visually evoked responses in 65% of recipient rats brains,[123] although the underlying mechanism producing this effect was not known.

Initial studies involving the transplantation of human fetal retinal tissue into rat hosts focused on the optimisation of transplant procedures as well as assessing graft host interaction.[124–127] Results from these studies showed that the development of human retinal transplants appears to parallel normal *in utero* development. Transplanted cone, rod and Muller cells all expressed cell-specific proteins, contained essential proteins for processing light and developed to maturity comparable to their normal counterparts.[124,125] In addition Little and co-workers[128] successfully transplanted human fetal RPE into rats suffering from genetically inherited retinal degeneration. Results showed a dramatic rescue effect with the number of observed photoreceptor nuclei being significantly greater in transplanted hosts than in sham-injected controls.[128] Results obtained in this study showed for the first time that transplanted human fetal RPE was able to rescue photoreceptor cells in a model of hereditary retinal degeneration.

With such promising results obtained in animal models, Kaplan *et al*.[129] conducted a feasibility and safety study of photoreceptor transplantation in patients suffering from retinitis pigmentosa. Sheets of human photoreceptor cells were harvested from cadaveric eyes using the vibratome-section method mentioned earlier,[108] and transplanted into the subretinal space of two patients with retinitis pigmentosa with a visual acuity of no light perception. Although 12 months later the visual acuity of no light perception remained in both patients, there appeared to be no signs of tissue rejection despite the patients not being immunosuppressed for this period.[129] In addition, cystoid macular oedema, uveitis and macular pucker were not observed.

Recently, Humayun and colleagues[130] conducted a human pilot study of human fetal retinal transplantation, with three specific aims: (1) to determine a safe surgical procedure for transplantation, (2) to observe whether transplanted tissue would be accepted into the subretinal space, (3) to see whether there was any improvement in vision-impaired recipients. Results showed that none of the recipients developed retinal vasculitis or intraocular inflammation after transplant and no rejection of the tissue was observed. Despite successful grafting procedures, only three patients demonstrated any improvement in light sensitivity in the initial months of follow-up. This appeared to be a transient effect as light sensitivity disappeared 3–13 months post-transplantation.[130]

Stem cell therapy of ocular disease

Despite significant advances in the transplantation field, progress has been limited with regard to its lack of ability to significantly improve sight quality.[129,130] Thus parallel studies have been conducted into the potential use of stem cells to treat retinal degeneration. Most tissues contain some stem cells that are capable of generating intermediate progenitors which then proliferate and differentiate into multiple yet distinct cell lineages.[2] Although found in tissues, the bone marrow remains the primary site where stem cells undergo self-renewal and differentiation. Stem cells can also be derived from immature embryos which are capable of differentiating into all somatic cell types[4] and those derived from adult tissue.[5,131] Stem cell therapy holds a particular advantage over transplantation methods since the procedure is autologous in nature. As a result it bypasses the limitations currently seen with transplantation methods, which include the risk of tissue rejection and the transmission of prion proteins.

Since stem cells can be isolated from a number of different origins, there exists a number of obstacles that could limit the potential use of these cells as part of a therapeutic regimen for disease. Currently there are major ethical and religious issues in the use of stem cells derived from fetal tissue. Many countries, including Japan and Australia, have introduced legislation that currently prohibits or will prohibit the derivation and use of human embryonic stem cells, whereas other countries, including the United Kingdom, Germany and the United States, appear more receptive.[132] Conversely, there appears a more positive consensus in the use of adult-derived stem cells for therapeutic use. Since in many disease settings stem cells cannot be isolated from the tissue of choice, alternative donor sites need to be investigated. Thus, bone-marrow-derived stem cells become a realistic and extremely viable option as the cells of choice.

Limbal stem cells

Stem cells are already being used in clinical practice for certain ocular surface diseases including pterygium. These stem cells are isolated from the limbal basal epithelium, which have been observed to contain the least differentiated cells of the corneal epithelium and have been commonly term limbal stem cells (LSCs). In addition there appears to be potential application of the use of LSCs in conditions when the stromal microenvironment is insufficient to support stem cell function, such as aniridia, or when stem cell deficiency occurs as a result of external factors that destroy the LSCs such as chemical or thermal injuries, ultraviolet and ionising radiation, Stevens–Johnson syndrome, advanced ocular cicatricial pemphigoid, multiple surgeries or excessive microbial infection.[133–140]

LSC transplantation is the treatment method of choice when stem cell deficiency affects the whole corneal surface. The first human trials of limbal transplantation were conducted by Kenyon and Tseng[141] who performed a limbal autograft transplantation to treat unilateral ocular surface disorders. In this particular study, both conjunctiva and limbus (including LSCs) were excised from the good eye and transplanted into the recipient eye.[141] Studies since this initial investigation have reported several variations of this limbal transplantational method, with most observing good reconstitution of the corneal epithelium and regression of neovascularisation.[138,139,142–145]

Although in principle the techniques used in limbal transplantation are similar, the source for donor cells can vary. Donor tissue can be obtained from the good eye (limbal autografts) when used to treat cases of unilateral disease[141,146–148] or can be obtained from a living related donor or cadaver (limbal allograft) when both eyes are affected.[136,149,150] One major limitation to the use of limbal allografts has been the high immunogenic stimulus of the transplant itself that ultimately leads to allograft rejection.[151–153] Thus, a number of studies have incorporated immunosuppressive agents with relative success,[154–157] although there is the implication that long-term systemic immunosuppression is required.

Retina-derived stem cells

To date most studies that have investigated the potential of stem cells for the treatment of other ocular diseases have focused on the isolation of progenitor cells from the tissue of choice, in this case from both embryonic and

adult retina.[26,158–162] Isolation has involved the excision of both the optic nerve and mesenchymal tissue to prevent contamination with brain tissue, the separation of the retina from the RPE and the removal of the central portion of the retina connected to the optic nerve.[131,158,161,162] Retinal cells are then dissociated into single cell suspensions and cultured under standard aseptic conditions. The retinal stem cells have then been successfully differentiated into ganglion cells,[163] amacrine cells,[162,163–165] bipolar cells[163] and both rod and cone photoreceptors.[26,158–160,166–170]

Studies since have focused on the successful transplantation of these stem cells into both normal and diseased retinas to observe and determine stem cell fate. Chacko and colleagues[171] investigated the survival and differentiation of cultured retinal progenitor cells upon subretinal transplantation. When transplanted either as neural spheres or as a cell suspension, it was observed that the progenitor cells survived without disrupting the host retina morphology. In addition, transplanted retinal stem cells were found to express photoreceptor-specific markers, which suggests that these progenitor cells have the potential to differentiate into photoreceptors.

Work conducted by Kurimoto and co-workers[172] further investigated the successful transplantation of retinal stem cells *in vivo* using a retinal degeneration model. Results showed that when injected into normal retina, the stem cells integrated both with the outer nuclear layer of the host retina as well as into the inner retinal layers. Those that had integrated with the outer retinal layers were found to express recoverin, a marker for photoreceptors and cone bipolar cells, whereas those that migrated into the inner layers of the retina did not express recoverin.[174] When assessed in the retinal degeneration model, it was observed that the progenitor cells had migrated and integrated with the host retina, but in this setting were also able to express rhodopsin (a photoreceptor specific marker) as well as recoverin.[172] It was concluded from this study that retinal progenitor cells might be able to respond to cues in the local microenvironment that induce the appropriate differentiation of transplanted cells.

Inducers of photoreceptor differentiation

Inducing agents

In a number of the different studies, particular agents have been identified to induce partial stem cell differentiation into photoreceptor cells.[26,160,161,173,174] Work conducted by Altschuler and co-workers[161] characterised retinal-cell-conditioned medium (CM) and extracts as sources of factors that influence photoreceptor development. Of 30 compounds pharmacologically screened, only taurine, which is found in high levels in the retina and central nervous system, and two similarly structured compounds were found to induce photoreceptor development. An additional study by Kelley and colleagues[160] further examined the effect of retinoic acid on fetal retinal cultures *in vitro* as a result of previous work that found it to be present in the developing retina.[175] Results obtained in this study found that exogenous retinoic acid caused a dose-dependent, specific increase in the number of cells that differentiated into photoreceptors, as well as a dose-dependent increase in the number of cells that developed into amacrine cells.[160]

Initial developmental studies found that retinal cells express the high-affinity neurotrophin-3 (NT-3) receptors.[176] In two subsequent investigations,[173,177] NT-3 was also assessed for its effect on photoreceptor development both *in vitro* and *in vivo*. Results from both investigations found that NT-3 promoted the differentiation of embryonic chick retinal cultures, and was an essential intrinsic signal acting in early development *in vivo* to promote the differentiation and survival of many retinal neurons.[173,177]

A recent study conducted by Davis *et al*.[26] examined the effect of activin A on fetal rat retinal cultures, since a previous investigation reported its expression in the developing retina.[178] *In vitro* results showed that exposure of retinal cultures to activin A caused the progenitor cells to exit the cell cycle and differentiate into rod photoreceptors. This effect was observed to be dose-dependent and specific since other retinal neurons generated (e.g. amacrine cells) were not affected by activin A treatment. *In vivo* results also showed that mice with the homozygous deletion of the activin βA gene exhibit a specific decrease in the number of rod photoreceptors compared with their heterozygous or wild-type counterparts.[26]

In addition, other studies have found that the addition of certain growth factors, including transforming growth factor (TGFα), fibroblast growth factor (FGF) and epidermal growth factor (EGF), aid in the proliferation of retinal stem cells.[163,164] Although these agents have been found to increase the rate of photoreceptor induction, they have not been found to induce complete differentiation of all stem cells in culture.[26,159] Thus the heterogeneous nature of such cultures limits their application due to the difficulty of result interpretation.

Specific gene inducers

Members of the hedgehog family of proteins have been associated in the patterning of multiple tissues during embryogenesis, including the neural tube, limbs, bone and sex organs.[179] Studies on the *Drosophila* eye have observed that the hedgehog gene family, which encodes for secreted proteins, is involved in controlling the timing and rate of photoreceptor differentiation during development.[174,180,181] Further investigation conducted by Dominguez and Hafen[182] showed that secretion of *hedgehog* proteins from cells at the posterior disc margin was an absolute requirement for the differentiation of ommatidial precursor cells into photoreceptors.

In a recent study, Levine and co-workers[170] successfully cloned several members of the *hedgehog* family including rat *Sonic hedgehog (Shh)*, *Desert hedgehog (Dhh)* and *Indian hedgehog (Ihh)* from fetal rat retina and

adult rat RPE. Fetal retinal cultures were then incubated with a specifically produced N-terminal *Recombinant Sonic Hedgehog protein (SHH-N)* for a period of 3–12 days, and resulting cell phenotypes then assessed using immunohistochemical techniques. It was shown that exposure of retinal progenitor cells to *SHH-N* resulted in a 2- to 10-fold increase in the number of cells that differentiated into photoreceptors, whereas there was no change in the number of differentiated retinal ganglion cells or amacrine cells. Subsequent studies have since found that *Ihh* also promotes neuronal differentiation of murine spinal cord precursors.[183]

The paired-liked homeodomain transcription factor *CRX* (*c*one-*r*od homeobo*x*) has been implicated in photoreceptor gene expression and rod outer segment development.[184–186] This transcription factor belongs to the *OTD/OTX homeobox* gene family, and its expression is restricted to developing and adult retinal photoreceptors and cells within the pineal gland.[184,185,187] *In vitro* studies have found that it specifically binds to regulatory elements in the promoters of several photoreceptor-specific genes such as *rhodopsin* and in transient transfection assays found to transactivate these genes.[184,185,187] *In vivo* studies using a *CRX* knockout mouse model[188] have also shown *CRX* to be essential for retinal development. Furthermore, mutations in the *CRX* gene have been associated with several retinal diseases, including autosomal dominant cone–rod dystrophy,[187,189,190] retinitis pigmentosa[192] and Leber congenital amaurosis (LCA).[191–193]

With the advancement of successful stem cell isolation, recent work conducted by Haruta and colleagues[194] investigated the effect of this gene on stem cell differentiation. Retinal stem cell from 3- to 4-week-old rats were isolated and cultured with basic fibroblast growth factor (bFGF) and infected with replication-deficient adenovirus encoding *CRX*. Using immunohistochemical techniques, results showed that adenovirus-mediated gene transfer of *CRX* notably promoted retinal stem cell differentiation into photoreceptors.[194]

Potential application for MSC application for ocular disease

To date no studies have investigated the potential of MSCs as a source of photoreceptor progenitor cells *in vitro* or *in vivo*. These cells have been shown to possess great multilinear potential, differentiating into osteogenic, chondrogenic, adipogenic, hepatic, glial and muscle cell lineages.[8,10–12,15–17] MSCs hold additional advantages over current methods in that the isolation procedure is less labour-intensive, only small volumes are required at harvesting, and the cells are easy to culture under standard conditions. The fact that there are significant ethical and moral issues with using human fetal tissue further emphasises the enormous potential of these cells in both cell and gene therapy.

Although there exists enormous potential in using MSCs in the formation of non-haematopoietic cell types, there are currently a number of obstacles, including the heterogeneity of differentiated stem cell populations, that could limit its practical application. As a result alternative avenues by which to derive specifically desired cell lineages, such as photoreceptor using MSCs, are currently being explored.

Stem cell plasticity

Marrow stromal cells

One such avenue is the specific induction of retinal photoreceptors from progenitor cells isolated from sites other than the optical retina and bone marrow, such as the brain. Initial studies by Kopen and co-workers[4] showed that murine MSCs were able to be engrafted into neonatal brain, and were shown to differentiate into astrocytes and neurones. A subsequent investigation by Woodbury and colleagues[23] showed that both rodent and human MSCs were capable of differentiating into neurones *in vitro*, when exposed to a specifically formulated neuronal induction medium. Resulting differentiated MSCs were shown to exhibit a neuronal phenotype, expressing neurone-specific enolase (NSE), HeuN, neurofilament-M (NF-M) and tau.[23] In a similar study Sanchez-Ramos and co-workers[17] also successfully differentiated murine and human MSCs into neural cells *in vitro* by using a specifically derived induction medium. Resulting cells were found to express the protein and mRNA for the neural cell markers: nestin, glial fibrillary protein (GFAP) and neurone-specific nuclear protein (NeuN).

Neural stem cells

Takahashi and co-workers[195] were the first to observe that neural progenitor cells isolated from adult rat hippocampus exhibit vast plasticity when grafted into the optic retina. Results indicated that the progenitor cells formed a non-disruptive lamina layer, and at 4 weeks post-grafting exhibited the morphologies of and positions of Muller, amacrine, bipolar, horizontal, photoreceptor and astroglial cells.[195] Despite this, results also showed that although still expressing neuronal or glial markers, none of the progenitors cells acquired end-stage markers uniquely found in retinal cells. Since neural cells have been successfully engrafted into the optic retina but appear not to disrupt its complex architecture, as well as partially differentiating into photoreceptor cells, these progenitor cells in particular may hold potential as a progenitor pool source for retinal cells.

Additional studies since have further explored this possibility by examining neural stem cell fate when transplanted into particular retinal degenerational models. Work by Lauritzen and colleagues[196] determined whether retinal degenerational mice (S334ter) were suitable recipients for adult rat hippocampal progenitor cell transplantation. In this

study, the neural stem cells were transplanted into the intravitreal space of recipient mice and assessed over a 4 week post-transplantational period. Results showed that there was no evidence of endophthalmitis or inflammation as a result of the transplantational procedure and that grafted cells integrated with host retinas. In particular it was observed that there was a greater level of neural stem cell integration in S334ter mice than in control animals.[196]

Young *et al.*[197] conducted a similar study whereby adult rat hippocampal progenitor cells were isolated and cultured from Fischer 344 rats, and then transplanted into the eyes of rats with a genetic retinal degeneration. Animals were then monitored for a period of 4 months post-transplantation and neural stem cell fate was determined using immunohistochemical techniques. Results revealed that adult rat hippocampal progenitor cells extensively integrated with the host retina, and that after 18 weeks post-transplantation, grafted cells expressed mature neuronal markers including NF-200, map-5 and calbindin.[197] Similar levels of hippocampal-derived neural stem cell integration have been observed when transplanted into adult rat retina that were mechanically injured.[198]

In a similarly designed investigation, Mizumoto and co-workers[199] assessed the effects of transplanting human fetal neural stem cells into a retinal degenerational rat model (RCS). Human neural progenitor cells isolated and cultured from 17-week-old fetal donor tissue were transplanted into the vitreous cavity of RCS rats and then monitored over a 4 week post-transplantational period. The human neural progenitor cells survived transplantation and after a 2 week period underwent mature neuronal differentiation, as assessed by the detection of the neuronal-specific marker MAP.[199]

Retinal stem cells

Interestingly, two other recent investigations have studied the multilinear potential of photoreceptor cells themselves.[161,162] Ahmad and colleagues[162] first reported that isolated embryonic retinal progenitor cells were capable of differentiating into neural and glial cell lineages, after the removal of epidermal growth factor (EGF) from its microenvironment. In a subsequent study[162] this same group found that progenitor cells isolated from the pigmented ciliary bodies, although displaying retinal specific properties, were successfully differentiated into neural and glial cells. Although the retina originates from the neural tube it becomes regionally isolated and high specialised in early development. The fact that neural stem cells have been shown to develop retinal morphologies, and retinal cells neural and glial morphologies, only further emphasises the potential of these particular progenitor cells in specified cell lineage development.

Conclusion

As shown in this review bone marrow stromal cells play an important role in a number of physiological and pathological settings, being involved in cellular replacement and repair in response to injury. This review has summarised the current advances in this field to date, highlighting a number of areas where stem cells hold the potential to treat injury or disease. A number of recent studies mentioned in this review have shown the multilinear potential of MSCs outside haematopoietic lineages including hepatocyte, glial and muscle cell differentiation. This fact has enables researchers to contemplate the use of MSCs in the treatment of a whole array of disease settings including retinal degeneration. Despite their potential many limiting factors exist, including the fact that current methods have only been successful in obtaining a heterogeneous population of fully differentiated cells. In addition most of the target organs and tissues to be treated are composed of more than one fully differentiated cell type. Compounded to this is the complex structural array of the organ itself as well as the complex interactions that exist between the cells. Despite these obstacles, further investigations are essential to fully explore the great potential of MSC therapy.

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